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on February 13, 2002

MILTON L. HONIG
Reg. No. 28,617
Attorney for Applicant(s)

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: Husken et al.

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For: OIL HAVING INCREASED POLYPHENOL CONTENT

Edgewater, New Jersey 07020
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SUBMISSION OF PRIORITY DOCUMENT

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

Pursuant to rule 55(b) of the Rules of Practice in Patent Cases, Applicant(s) is/are submitting herewith a certified copy of the European Application No. 00204714.0 filed December 22, 2000, upon which the claim for priority under 35 U.S.C. § 119 was made in the United States.

It is respectfully requested that the priority document be made part of the file history.

Respectfully submitted,

Milton L. Honig
Reg. No. 28,617
Attorney for Applicant(s)

MLH/mt
(201) 840-2403

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Bescheinigung | Certificate | Attestation

Die angehefteten Unterlagen stimmen mit der ursprünglich eingereichten Fassung der auf dem nächsten Blatt bezeichneten europäischen Patentanmeldung überein.

The attached documents are exact copies of the European patent application described on the following page, as originally filed.

Les documents fixés à cette attestation sont conformes à la version initialement déposée de la demande de brevet européen spécifiée à la page suivante.

Patentanmeldung Nr. | Patent application No. | Demande de brevet n°

00204714.0

Der Präsident des Europäischen Patentamts;
Im Auftrag

For the President of the European Patent Office

Le Président de l'Office européen des brevets
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R C van Dijk

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THE HAGUE, 05/12/01
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Blatt 2 der Bescheinigung
Sheet 2 of the certificate
Page 2 de l'attestation

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Demandeur(s):
UNILEVER N.V.
3013 AL Rotterdam
NETHERLANDS

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Oil having increased polyphenol content

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Oil having increased polyphenol content

The present invention relates to a method for increasing the polyphenol content of an oil and to the oils obtained with such a method.

The present invention relates to oils, in particular vegetable oils and food products containing a certain amount of fat or oil, such as spreads, mayonnaises, salad dressings and sauces. Fats and oils form a substantial part of the average human food consumption. Since fat consumption is associated with an increased risk of cardiovascular disorders, the nutritional value of different types of fat as well as methods for reducing the amount of fat in food products has been the object of extensive investigation.

Recently, also the nature and the effects on health of fat attributes, the so-called minor nutrients which are present in small amounts in non-refined natural fats is subject of such investigations. It has been found that the minor nutrients which are denoted as anti-oxidants, including fat polyphenols, positively interfere with the body's cardiovascular system. Polyphenols are compounds which share a phenolic hydroxyl group. Usually polyphenols are present not as a single compound but as a mixture of different polyphenols. One of the sources of polyphenols are olives. Olive fruit originating polyphenols are for example oleuropein, aglycons, tyrosol or hydroxytyrosol.

Traditionally, most natural fats are refined before they are used as an ingredient for the preparation of food. However, traditional fat refining is not discriminating to the nature of the fat ingredients and aims at the removal of all substances

other than triglycerides, including minor nutrients. For instance, Lampante olive oil, is deodorized at temperatures of 240 °C or even more. Under such conditions also the oil's valuable minor nutrients, including natural anti-oxidants, particularly the typical olive oil polyphenols are almost completely stripped.

Thus, the object of the present invention is the addition of antioxidants such as polyphenols, included in olive fruits to (refined) oils and fat based products such as spreads, mayonnaise, salad dressings and sauces. A further object is to increase the level of such antioxidants in an oil without deterioration of colour and taste of the oil.

These and other objects are attained by the method of the present invention, which comprises the steps of contacting the oil with olive fruit material in the presence of an acid and separating the oil from the olive fruit material.

By means of this method the beneficial antioxidant components present in olive fruit material are released from the olive fruit material and extracted into the oil. After separation of the olive fruit material a clear oil is obtained having good taste and color properties and an increased polyphenol level.

The acid to be used for this method is in particular hydrochloric acid or a food grade acid such as citric acid, phosphoric acid, acetic acid, lactic acid, ascorbic acid or any other food grade acid. The acid is added to the mixture of oil and olive fruit material as a concentrated aqueous solution, for instance containing more than 30 % (w/w) acid. The amount of acid added is 0.1 to 30 wt. %, preferably 0.5 to 5 wt.%,

based on pure acid and the weight of oil and olive fruit material.

The olive fruit material can be whole olive fruits, olive fruit particles or olive residue. With olive residue is meant the residue that remains after production of olive oil by malaxation of olive fruits. Such a residue usually has a water content of about 50 to 70 wt.% and can have a polyphenol content of e.g. 2000 to 30,000 ppm (wt/wt). The polyphenol content varies for instance depending on the ripeness or origin of the olives. The amount of olive fruit material in the oil is 0.1 to 50 wt.%, preferably 10 to 40 wt.%, based on the weight of the oil.

The temperature at which the oil is contacted with the olive fruit material is at least 10 °C. However, the method according to the invention is preferably carried out at elevated temperatures. This increases the amount of polyphenols transferred to the oil. Preferably, the oil is contacted with the olive fruit material at a temperature of at least 50 °C, more preferably at least 70 °C, most preferably 90 to 100 °C. The optimal time for contacting oil and olive fruit material can be determined by a skilled person. In general this period will be at least 30 minutes, preferably at least 90 minutes. Preferably the mixture of oil and olive fruit material is stirred during the contact time. Increasing the stirring rate will increase contact area and thus mass transfer of the polyphenols to the oil.

Preferably, the method according to the present invention is used to fortify vegetable oils. Examples of vegetable oils which can be fortified according to the invention are olive oil, rapeseed oil, sunflowerseed oil, soybean oil and corn oil. Preferably

olive oil is fortified. The invention is not limited to fortification of oils which are devoid of any polyphenol, either by nature or because of a refining process, but also of oils which contain polyphenols of their own such as (extra) virgin olive oils. Examples of other olive oils which can be fortified according to the present invention are an extra virgin olive oil, a fine virgin olive oil, a semi-fine or regular virgin olive oil, a refined virgin olive oil, such as a Lampante oil, or an olive residue oil but also an olive oil blend, which contains part virgin olive oil and part refined olive oil.

The present invention thus also relates to the oil obtained with the above described method. The oil will have a polyphenol content of more than 150 ppm. The total content of polyphenols in oil can be established by standard methods, e.g. by the colorimetric Gutfinger method as described in J.Am.Oil.Chem.Soc. 1981, 11, pp. 966-968, which method is based on the reaction of a methanolic extract of olive oil and the Folin-Ciocalteau reagent. Polyphenol content can also be determined by HPLC. Another characteristic of the oil of this invention is that it is a clear oil, even though it contains a high amount of polyphenols. In particular the invention provides a clear, pure olive oil having a polyphenol content higher than 150 ppm, in particular having a caffeic acid content higher than 150 mg/kg oil..

The present invention also relates to food products containing the fortified oil. These food products can be mixtures of the fortified oil and another oil, but also products such as a spread, salad dressing, mayonnaise or sauce. Spreads are food compositions which usually contain a substantial amount of fat, often 40 wt.% or more. Usually the fat consists of a liquid oil and a structuring fat which gives the fat blend a proper consistency. Sauces are meant to include any type of sauce, for

instance sauces that are ready to use, in particularly after having been heated, such as for instance tomato sauces. Processes for the manufacture of these products are well known in the art and need no illustration.

It goes without saying that the invention is not restricted to oils which only have a vegetable origin. Animal oils, such as fish oil, can also be used. It might be advantageous to use a fat blend which partly consists of animal fat and/or marine oils or fats derived from such fats/oils by fractionation or interesterification. Fat and oil are terms which are used interchangeably in this specification. The term oil is generally used when the fat is liquid at ambient temperature.

The invention will now be further described by means of the non-limiting examples and the attached figures, wherein

figure 1 shows the increase of polyphenols in refined olive oil using concentrated hydrochloric acid;

figure 2 shows the increase of polyphenols in refined olive oil using citric acid;

figure 3 shows a HPLC analysis of a sample according to example 3 after 30 minutes; and

figure 4 shows a HPLC analysis of a sample according to example 3 after 126 minutes and the addition of HCl.

Examples

Example 1

To 101.6 grams of olive residue, 205.5 grams of refined olive oil was added. The mixture was stirred mechanically and the temperature was subsequently raised to 95°C. Mixture was allowed to equilibrate. After 25 minutes a sample was taken and analysed for polyphenols using the Gutfinger method. Then 5 ml of a concentrated hydrochloric acid solution (37%) was added to the mixture. After several time intervals samples were taken

and subsequently analysed for polyphenols using the Gutfinger method. In order to remove solids and water from the samples, the samples are centrifuged at 3500 rpm for 30 minutes. Results of the Gutfinger analysis are reported in figure 1. Polyphenol content increases quickly after addition of concentrated hydrochloric acid.

Example 2

To 101.4 grams of olive residue, 206.1 grams of refined olive oil was added. The mixture was stirred mechanically and the temperature was subsequently raised to 95°C. Mixture was allowed to equilibrate. After 50 minutes and 80 minutes a sample was taken and analysed for polyphenols using the Gutfinger method. Then 10 ml of a citric acid solution (60% w/w) was added to the mixture. After several time intervals samples were taken and subsequently analysed for polyphenols using the Gutfinger method. In order to remove solids and water from the samples, the samples are centrifuged at 3500 rpm for 30 minutes. Results of the Gutfinger analysis are reported in figure 2. Polyphenol content increases quickly after addition of citric acid.

Example 3

To 154.8 grams of olive residue, 303.5 grams of refined olive oil was added. The mixture was stirred mechanically and the temperature was subsequently raised to 95°C. Mixture was allowed to equilibrate. After 30 minutes a sample was taken and analysed for polyphenols using the Gutfinger method and analysed for the polyphenols composition using the HPLC method. Then 7.5 ml of a concentrated hydrochloric acid solution (37%) was added to the mixture. After 126 minutes a samples was taken and subsequently analysed for polyphenols using the Gutfinger method and analysed for the polyphenols composition using the HPLC method. In order to remove solids and water from the

samples, the samples are centrifuged at 3500 rpm for 30 minutes. Results of the Gutfinger analysis are reported in table 1. The results of the HPLC analysis are reported in table 2 and the chromatograms are shown in figures 3 and 4.

For HPLC analysis the following method was used. The analytical separations were performed on a Waters 600 S liquid chromatograph equipped with a waters 616 pump and a waters 490 UV multiwavelength detector. Injection of the samples was carried out by a 10 ul Rheodyne sample loop. A chrompack 25 cm * 4.6 mm * ¼ inch Intersil5 ODS column was applied using a gradient flow rate of 1 ml/min. The elution system consisted of solvent A (2% acetic acid in water) and solvent B (methanol). Gradient: 0-20 min., A/B 85/15 %; 20-50 min., 15-75 B in A; 50-55 min., A/B 25/75, 55-56 min 75-100 B in A; 56-65 min., 100% B. UV was measured at 280 nm (for quantification) and 239 nm.

Table 1 Polyphenolic content

Sample	Polyphenolic content (mg/kg caffeic acid)
30 minutes at 95°C	146
126 minutes after adding acid	397

Table 2 HPLC results

Retention Time (minutes)	30 min. at 95°C (area)	126 min. after adding acid (area)	Component
4.04	21923		
6.65	8742	4178232	
7.76		135771	
9.27	128361	145286	Hydroxy tyrosol
10.92		63763	
14.88	36113	607732	
15.45		235714	
16.37	149375	1832740	Tyrosol
21.47		221384	
25.32	58590	241784	
27.67	128080	168907	
30.44		1023006	
31.70	903041	298597	
33.46	50968	3231294	
34.05	502165	716243	
35.37	247782	50434	
36.71	28473	82153	
37.25	190364	103863	
38.40	54189	1351964	
39.05	165608	98697	

39.92	6239	600276	
40.10	37375	28175	
40.96	520092	244570	Hydroxy tyrosol related aglycon
41.78	403506	28754	
42.50		84376	
42.83		380553	
43.50		19008	
43.94	59003	266661	
44.42	1745343	943163	Tyrosol related aglycon
45.60	124423		
45.30	81431		
45.80	55349	744871	Hydroxy tyrosol related aglycon
46.42	81431		
46.93	56294	189785	Hydroxy tyrosol related aglycon
47.46	21635	33105	Hydroxy tyrosol related aglycon
49.26	114016	183249	Tyrosol related aglycon
49.74	34295		
50.29	11411	87843	Tyrosol related aglycon
51.74	33161	1616271	
52.10		1616271	
52.37	19644	64896	
52.90		64896	
53.57		76154	

Claims

1. Method for increasing the polyphenol content in an oil, comprising the steps of contacting the oil with olive fruit material in the presence of an acid and separating the oil from the olive fruit material.

2. Method according to claim 1, wherein the acid is hydrochloric acid, citric acid, phosphoric acid, acetic acid, lactic acid or ascorbic acid.

3. Method according to claim 1 or 2, wherein the olive fruit material is selected from the group consisting of whole olive fruits, olive fruit particles and olive residue.

4. Method according to any of the preceding claims, wherein the oil is contacted with the olive fruit material at a temperature of at least 50 °C.

5. Method according to claim 4, wherein the temperature is at least 70 °C, preferably 90 to 100 °C.

6. Method according to any of the preceding claims, wherein the acid concentration is 0.1 to 30 wt. %, preferably 0.5 to 5 wt.%, based on the weight of oil and olive fruit material.

7. Method according to any of the preceding claims, wherein the oil is a vegetable oil.

8. Method according to claim 7, wherein the oil is an olive oil.

9. Oil obtainable by the method of any of the preceding claims, having a caffeic acid content higher than 150 mg/kg oil.

10. Food product containing an oil according to claim 9.

11. Food product according to claim 10, which is a mixture of a vegetable oil, preferably an olive oil and the oil according to claim 9 or 10.

12. Food product according to claim 10, which is a spread, mayonnaise, salad dressing or tomato sauce.

Abstract

The present invention provides a method for increasing the polyphenol content in an oil, comprising the steps of contacting the oil with olive fruit material in the presence of an acid and separating the oil from the olive fruit material. The acid is preferably hydrochloric acid, citric acid, phosphoric acid, acetic acid, lactic acid or ascorbic acid. The olive fruit material can be selected from the group consisting of whole olive fruits, olive fruit particles and olive residue.

Figure 1 Polyphenolic content increase of refined live il using c ncentrated hydrochloric acid

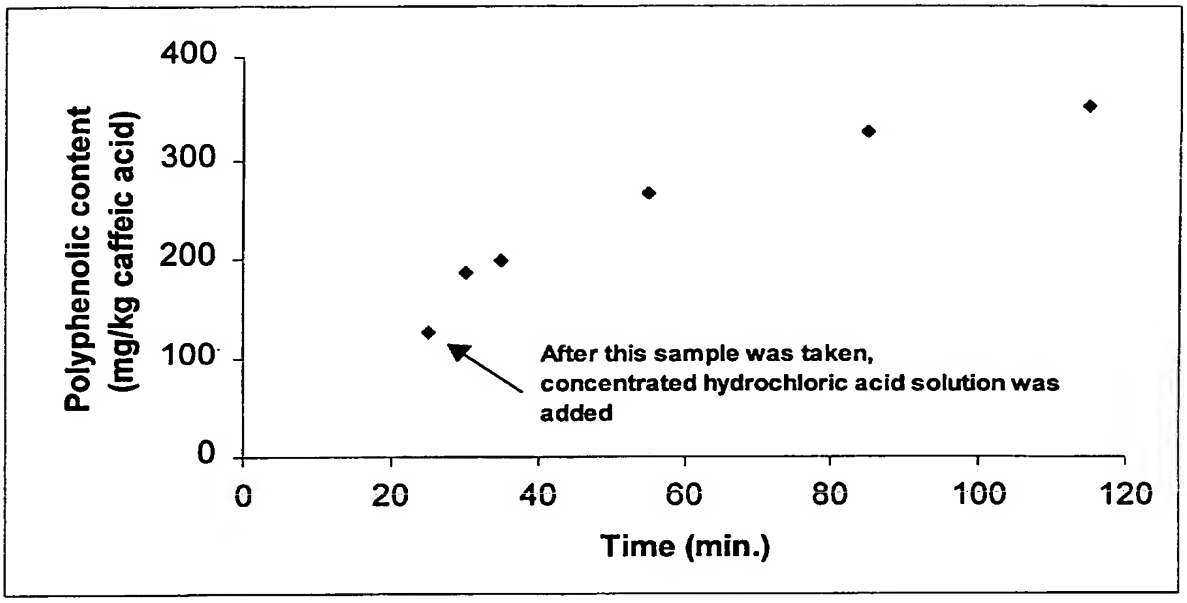

Figure 2 Polyphenolic content increase of refined olive oil using citric acid

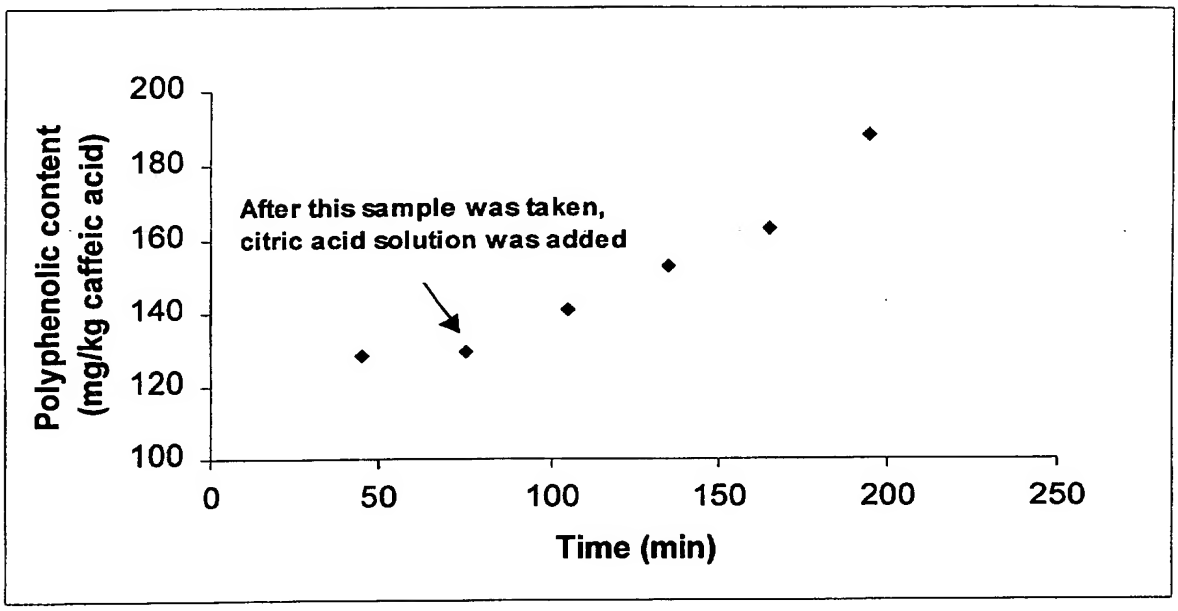

Figure 3 HPLC analysis of sampl taken after 30 minutes at 95°C

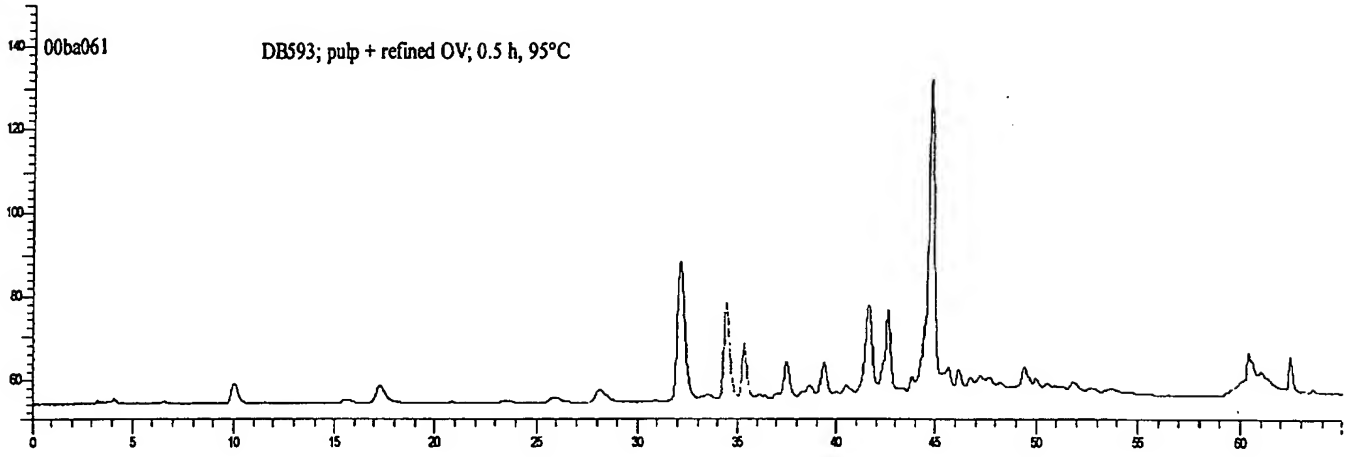

Figure 4 HPLC analysis of sample taken 126 minutes after addition of acid